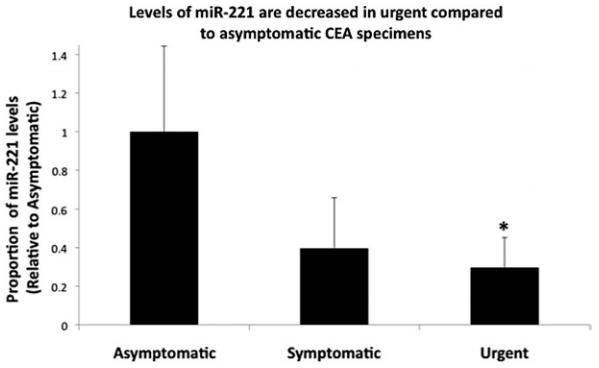

Fig.

possible fibrous cap degradation and rupture. *:P = .039.

Author Disclosures: H. A. Bazan: Nothing to disclose; **C. B. Coleman**: Nothing to disclose; **T. Smith**: Nothing to disclose; **W. Sternbergh III**: Nothing to disclose; **T. Woods**: Nothing to disclose.

C9k: Poster Session - Research (1)

PS194.

Role of PI3k/Akt Pathway in the Cytoprotective Effects of Erythropoietin and Derivatives in Ischemic Human Myotubes

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Objectives: Erythropoietin (Epo) has tissue-protective effects following ischemic injury, mediated through the EpoR-βcR heteroreceptor. Having previously shown presence of the EpoR in human skeletal muscle, we now aim to show the direct interaction of the EpoR with the βcR. Further, we wanted to determine the cytoprotective effects of Epo and an Epo-derivative (ARA-290) in a human in vitro model of skeletal muscle and establish the role of PI3K/Akt pathway in protecting cells from apoptosis.

Methods: Gastrocnemius muscle biopsies were obtained from patients with critical limb ischemia and control samples were obtained from non-ischemic patients. Co-immunoprecipitation (Co-IP) was performed to demonstrate heterodimerisation of EpoR with βcR. Human myoblasts were isolated to determine the cytoprotective effects of Epo and ARA-290 pre-treatment on myotubes subjected to simulated ischemia. Wortmannin (PI3k inhibitor) was used to determine the role of PI3k/Akt pathway in mediating cytoprotection. Western blot analysis, using the pro-apoptotic marker cleaved caspase-3 was performed and compared with levels of Akt and phosphorylated-Akt, using western blot analysis.

Results: EpoR and βcR has been demonstrated in human skeletal muscle. Heterodimerisation of EpoR-βcR was confirmed by Co-IP. Epo and ARA-290 were able to ameliorate the ischemia-induced apoptosis on isolated human myotubes. Addition of wortmannin, to ARA-290 or Epo pre-treated cells, abolished the reduction in apoptosis. Further, a reduction in apoptosis was associated with an increase in phosphorylated-Akt on western blot analysis.

Conclusions: EpoR-βcR heteroreceptor was demonstrated in human skeletal muscle. ARA-290 attenuates apoptosis in ischemic human myotubes suggesting a potential role in tissue protection during skeletal muscle injury. We propose that the PI3k/Akt signalling pathway is involved in mediating this cytoprotection.

Author Disclosures: D. Baker: Nothing to disclose; **G. Hamilton**: Nothing to disclose; **J. Tsui**: Nothing to disclose; **R. Yu**: Nothing to disclose.

PS196.

Tracking the Migration of Porcine Mesenchymal Stem Cells with the MRI Contrast Agent Ferex in an AAA Model

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Objectives: Mesenchymal stem cells (MSC) are being investigated in a porcine abdominal aortic aneurysm (PAAA) model for their repair potential. This study uses MSCs labeled with the MRI contrast agent Ferex to non-invasively evaluate MSC migration in-vivo.

Methods: MSCs from six pigs were isolated from bone marrow via Ficoll Paque separation and expanded in culture. Using a Lentiviral vector, MSC from all six pigs were labeled with green florescent protein (GFP). MSCs from four of these pigs were labeled with Ferex using Poly-L-Lysine, a cationic transfecting agent. These cells were analyzed for Ferex uptake and viability. Preservation of the MSC phenotype was confirmed using cytometry by detecting positive CD90 signals and negative CD45 and CD117. Transmission electron microscopy established that Ferex particles localized to lysosomes of labeled cells. MSCs were then injected into the PAAA. In-vivo MRI was performed at intervals followed by euthanasia and histologic analysis.

Results: Ferex labeled MSCs were visible at 4, 11, and 15 days using MRI and the signal loss progressed at each study interval representing cellular movement (Fig 1). MSC migration and localization were confirmed with GNP visualization on fluorescence microscopy and immunohistochemistry. A correlation between in-vivo MRI signals and iron deposition was clearly demonstrated histologically using Perl's staining.